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<b>(54) Title:</b> DNA SEQUENCES AND SECRETED PROTEINS ENCODED THEREBY  <b>(57) Abstract</b>  Novel polynucleotides and the proteins encoded thereby are disclosed.		

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## **DNA SEQUENCES AND SECRETED PROTEINS ENCODED THEREBY**

5           This application claims priority from application Ser. No. 08/514,014, filed on August 11, 1995, which was converted to provisional application Ser. No. 60/ \_\_\_\_\_ on July 19, 1996.

### **FIELD OF THE INVENTION**

10           The present invention provides novel polynucleotides and proteins encoded by such polynucleotides, along with therapeutic, diagnostic and research utilities for these polynucleotides and proteins.

### **BACKGROUND OF THE INVENTION**

15           Technology aimed at the discovery of protein factors (including e.g., cytokines, such as lymphokines, interferons, CSFs and interleukins) has matured rapidly over the past decade. The now routine hybridization cloning and expression cloning techniques clone novel polynucleotides "directly" in the sense that they rely on information directly related to the discovered factor (i.e., partial DNA/amino acid  
20           sequence of the factor in the case of hybridization cloning; activity of the factor in the case of expression cloning). More recent "indirect" cloning techniques such as signal sequence cloning, which isolates DNA sequences based on the presence of a now well-recognized secretory leader sequence motif, as well as various PCR-based or low stringency hybridization cloning techniques, have advanced the state of the art by  
25           making available large numbers of DNA/amino acid sequences for factors that are known to have biological activity by virtue of their secreted nature in the case of leader sequence cloning, or by virtue of the cell or tissue source in the case of PCR-based techniques. It is to these factors and the polynucleotides encoding them that the present invention is directed.

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SUMMARY

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 38 to nucleotide 1447;
- 10 (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:1 encoding a protein having biological activity;
- (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2;
- 15 (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity;
- (e) a polynucleotide which is an allelic variant of SEQ ID NO:1; and
- (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

20

In another embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 52 to nucleotide 2034;
- (b) a polynucleotide comprising a fragment of the nucleotide  
25 sequence of SEQ ID NO:3 encoding a protein having biological activity;
- (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:4;
- (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity;
- 30 (e) a polynucleotide which is an allelic variant of SEQ ID NO:4; and
- (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

35 In another embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- 5 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 76 to nucleotide 474;
- (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:5 encoding a protein having biological activity;
- (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6;
- 10 (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity;
- (e) a polynucleotide which is an allelic variant of SEQ ID NO:5; and
- 15 (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

In another embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 67 to nucleotide 348;
- 20 (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:7 encoding a protein having biological activity;
- (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:8;
- 25 (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 having biological activity;
- (e) a polynucleotide which is an allelic variant of SEQ ID NO:7; and
- (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).
- 30

In another embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 75 to nucleotide 356;
- 35 (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:9 encoding a protein having biological activity;

5 (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:10;

(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity;

10 (e) a polynucleotide which is an allelic variant of SEQ ID NO:9; and

(f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

In another embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

15 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 86 to nucleotide 544;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:11 encoding a protein having biological activity;

20 (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:12;

(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity;

(e) a polynucleotide which is an allelic variant of SEQ ID NO:11; and

25 (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

In certain preferred embodiments, the polynucleotide is operably linked to an expression control sequence. The invention also provides a host cell, including bacterial, yeast, insect and mammalian cells, transformed with such polynucleotide compositions.

Processes are also provided for producing a protein, which comprise:

(a) growing a culture of the host cell transformed with such polynucleotide compositions in a suitable culture medium; and

(b) purifying the protein from the culture.

35 The protein produced according to such methods is also provided by the present invention.

5 Compositions comprising a protein biological activity are also disclosed. In preferred embodiments the protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:2;
- (b) fragments of the amino acid sequence of SEQ ID NO:2;
- 10 (c) the amino acid sequence of SEQ ID NO:4;
- (d) fragments of the amino acid sequence of SEQ ID NO:4;
- (e) the amino acid sequence of SEQ ID NO:6;
- (f) fragments of the amino acid sequence of SEQ ID NO:6;
- (g) the amino acid sequence of SEQ ID NO:8;
- 15 (h) fragments of the amino acid sequence of SEQ ID NO:8;
- (i) the amino acid sequence of SEQ ID NO:12; and
- (j) fragments of the amino acid sequence of SEQ ID NO:12;

the protein being substantially free from other mammalian proteins.

Such compositions may further comprise a pharmaceutically acceptable carrier.

20 Compositions comprising an antibody which specifically reacts with such protein are also provided by the present invention.

Methods are also provided for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition comprising a protein of the present invention and  
25 a pharmaceutically acceptable carrier.

#### BRIEF DESCRIPTION OF FIGURES

Fig. 1 is an autoradiograph evidencing the expression of clone J5 in COS cells (indicated by arrows). J5 is processed into multiple bands, with the major band at  
30 approximately 58 kD.

Fig. 2 is an autoradiograph evidencing the expression of clone L105 in COS cells (indicated by arrows).

Fig. 3 is an autoradiograph evidencing the expression of clone H174 in COS cells (indicated by arrows).

35 Fig. 4 is an autoradiograph evidencing the expression of clone B18 in COS cells (indicated by arrows).

DETAILED DESCRIPTIONISOLATED PROTEINS AND POLYNUCLEOTIDES

The sequence of a polynucleotide encoding one protein of the present invention is set forth in SEQ ID NO:1, with the coding region extending from nucleotides 38 to 1447. This polynucleotide has been identified as "clone J5". The amino acid sequence of the protein encoded by clone J5 is set forth in SEQ ID NO:2. Clone J5 was deposited with the American Type Culture Collection on August 11, 1995 and given the accession number ATCC 69885. SEQ ID NO:1 represents a spliced combination of sequence obtained from an isolated clone identified as "J5\_3\_fl", with additional 5' sequence obtained from a second double stranded clone. Clone J5 was isolated from a human activated peripheral blood mononuclear cell (PBMC) library using a trap which selects for nucleotides encoding secreted proteins; therefore, clone J5 does encode a secreted factor. J5 encodes a novel protein; BLASTN/BLASTX or FASTA searches revealed no exact sequence matches. However, a BLASTX search revealed homology between the J5 protein (in the approximate region of amino acids 62-129 of SEQ ID NO:2), epididymal apical proteins (including without limitation, epididymal apical protein I-precursor (*Macaca fascicularis*) (accession X66139)) and several snake venom haemorrhagic peptides (disintegrins) (including without limitation those assigned accession U01235-1237, X68251, and M89784). Analysis of the full-length J5 sequences revealed that the disintegrin domain was incomplete and that this clone did not contain an EGF-domain, as with some of the other disintegrin family members. J5 does contain a conserved metallo-proteinase domain. Based upon these homologies, J5 and these homologous proteins are expected to share at least some activities.

The sequence of a polynucleotide encoding another protein of the present invention is set forth in SEQ ID NO:3, with the coding region extending from nucleotides 52 to 2034. This polynucleotide has been identified as "clone J422". The amino acid sequence of the protein encoded by clone J422 is set forth in SEQ ID NO:4. Clone J422 was deposited with the American Type Culture Collection on August 11, 1995 and given the accession number ATCC 69884. SEQ ID NO:3 represents a spliced combination of sequence obtained from an isolated clone



5 identified as "J422\_fl", with additional 5' sequence obtained from a second double  
stranded clone. Clone J422 was isolated from a human activated peripheral blood  
mononuclear cell (PBMC) library using a trap which selects for nucleotides encoding  
secreted proteins; therefore, clone J422 does encode a secreted factor. J422 encodes  
a novel protein; BLASTN/BLASTX or FASTA searches revealed no exact sequence  
10 matches. However, a FASTA search revealed homology between the J422 protein (in  
the approximate region of amino acids 34-156 of SEQ ID NO:4) and a number of  
*Drosophila* leucine-rich repeat (LRR) proteins. Analysis of the full-length J422  
sequences revealed that the conserved EGF-domain found in a number of LRR family  
members was not present in J422. Based upon these homologies, J422 and these  
15 homologous proteins are expected to share at least some activities.

The sequence of a polynucleotide encoding another protein of the present  
invention is set forth in SEQ ID NO:5, with the coding region extending from  
nucleotides 76 to 474. This polynucleotide has been identified as "clone L105". The  
amino acid sequence of the protein encoded by clone L105 is set forth in SEQ ID  
20 NO:6. Clone L105 was deposited with the American Type Culture Collection on  
August 11, 1995 and given the accession number ATCC 69883. Clone L105 was  
isolated from a murine adult thymus library using a trap which selects for nucleotides  
encoding secreted proteins; therefore, clone L105 does encode a secreted factor. L105  
encodes a novel protein; BLASTN/BLASTX or FASTA searches revealed no exact  
25 sequence matches. However, a BLASTX search revealed homology between the L105  
protein (particularly in the approximate region of amino acids 73-91 of SEQ ID  
NO:6), various monocyte and other chemoattractant proteins (including without  
limitation those assigned accession M577441, X71087, X72308, X14768 and  
M24545) and a chicken (*Gallus gallus*) cytokine (accession L34553). Based upon  
30 these homologies, L105 and these homologous proteins are expected to share at least  
some activities.

The sequence of polynucleotides encoding another protein of the present  
invention is set forth in SEQ ID NO:7 and SEQ ID NO:9, with the coding regions  
extending from nucleotides 67 to 348 and nucleotides 75 to 356, respectively. These  
35 polynucleotides have been identified as "clone H174-10" and "clone H174-43",  
respectively (collectively referred to herein as "H174"). The amino acid sequence of

5 the protein encoded by clones H174 is set forth in SEQ ID NO:8 and SEQ ID NO:10. Clone H174 was deposited with the American Type Culture Collection on August 11, 1995 and given the accession number ATCC 69882. Clones H174 were isolated from a human activated peripheral blood mononuclear cell (PBMC) library using a trap which selects for nucleotides encoding secreted proteins; therefore, H174 does encode  
10 a secreted factor. H174 encodes a novel protein; BLASTN/BLASTX or FASTA searches revealed no exact sequence matches. However, a BLASTX search revealed homology between the H174 protein, human IP-10 (accession M33266) and murine CRG-2 (accession M86820) (species homologs). Based upon these homologies, H174 and these homologous proteins are expected to share at least some activities.

15 The sequence of a polynucleotide encoding another protein of the present invention is set forth in SEQ ID NO:11, with the coding region extending from nucleotides 86 to 544. This polynucleotide has been identified as "B18". The amino acid sequence of the protein encoded by clone B18 is set forth in SEQ ID NO:12. Clone B18 was deposited with the American Type Culture Collection on July 6, 1995  
20 and assigned accession number ATCC 69868. Clone B18 was isolated from a human activated peripheral blood mononuclear cell (PBMC) library using a trap which selects for nucleotides encoding secreted proteins; therefore, clone B18 does encode a secreted factor. B18 encodes a novel protein; BLASTN/BLASTX or FASTA searches revealed no exact sequence matches. However, a BLASTX search revealed that the  
25 region from amino acid 29 to amino acid 163 of B18 (SEQ ID NO:12) shows marked homology to portions of murine CTLA-8 (amino acids 18 to 150, accession L13839) and herpesvirus *Saimiri* ORF13 ("herpes CTLA-8") (amino acids 19 to 151, accession X64346). Based upon these homologies, B18 is believed to be the human homolog of murine and herpes CTLA-8 (i.e., "human CTLA-8"). B18 may demonstrate  
30 proinflammatory activity, particularly in development of T-cell dependent immune responses. B18 is also expected to possess other activities specified herein.

Clones J5, L105, H174 and B18 were each transfected into COS cells labelled with <sup>35</sup>S-methionine and protein was expressed. Autoradiographs evidencing expression of the proteins in conditioned media are presented in Figs. 1, 2, 3 and 4,

5        respectively. The bands of protein expressed from the relevant clone are indicated by arrows.

Polynucleotides hybridizing to the polynucleotides of the present invention under stringent conditions and highly stringent conditions are also part of the present invention. As used herein, "highly stringent conditions" include, for example, at least  
10        about 0.2xSSC at 65°C; and "stringent conditions" include, for example, at least about 4xSSC at 65°C or at least about 50% formamide, 4xSSC at 42°C. Allelic variants of the polynucleotides of the present invention are also encompassed by the invention.

Fragments of the proteins of the present invention which are capable of exhibiting biological activity are also encompassed by the present invention.  
15        Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as described in H.U. Saragovi, *et al.*, Bio/Technology 10, 773-778 (1992) and in R.S. McDowell, *et al.*, J. Amer. Chem. Soc. 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as immunoglobulins for many purposes, including increasing  
20        the valency of protein binding sites. For example, fragments of the protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin. For a bivalent form of the protein, such a fusion could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may also be used to generate such fusions. For example, a protein - IgM fusion would generate a decavalent form of the protein  
25        of the invention.

The isolated polynucleotide of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman *et al.*, Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many suitable expression control sequences are known in the  
30        art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed  
35        (transfected) with the ligated polynucleotide/expression control sequence.

5 A number of types of cells may act as suitable host cells for expression of the protein. Mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue,  
10 primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells.

Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins.  
15 Potentially suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments  
20 may be accomplished using known chemical or enzymatic methods.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in  
25 kit form from, e.g., Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

30 The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (i.e., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an  
35 affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or

5 Cibacrom blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion  
10 protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX). Kits for expression and purification of such fusion proteins are commercially available from New England BioLab (Beverly, MA), Pharmacia (Piscataway, NJ) and InVitrogen, respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed  
15 to such epitope. One such epitope ("Flag") is commercially available from Kodak (New Haven, CT).

Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the  
20 protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

The protein of the invention may also be expressed as a product of transgenic  
25 animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

The protein may also be produced by known conventional chemical synthesis. Methods for constructing the proteins of the present invention by synthetic means are  
30 known to those skilled in the art. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic  
35 compounds and in immunological processes for the development of antibodies.

5           The proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications in the peptide or DNA sequences can be made by those skilled in the art using known techniques. Modifications of interest in the protein sequences may include the replacement, insertion or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Mutagenic techniques for such replacement, insertion or deletion are well known to those skilled in the art (see, e.g., U.S. Patent No. 4,518,584).

10           Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and may thus be useful for screening or other immunological methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are believed to be encompassed by the present invention.

20

#### **USES AND BIOLOGICAL ACTIVITY**

          The polynucleotides of the present invention and the proteins encoded thereby are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

25

#### **RESEARCH TOOL UTILITY**

30           The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers (when labeled) to map related gene positions; to compare with endogenous

35

5 DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise  
10 anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which  
15 binding occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used to raise antibodies or to elicit another immune response; as a reagent (including the labelled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding  
20 protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors  
25 of the binding interaction. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these "research tool" utilities are capable of being developed into reagent grade or kit format for commercialization as "research products."

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### **CYTOKINE AND CELL PROLIFERATION/DIFFERENTIATION ACTIVITY**

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting)  
35 activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited

5 activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2,  
10 CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: *Current Protocols in Immunology*, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and  
15 Wiley-Interscience (Chapter 3, *In Vitro* assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, *Immunologic studies in Humans*); Takai et al., *J. Immunol.* 137:3494-3500, 1986; Bertagnolli et al., *J. Immunol.* 145:1706-1712, 1990; Bertagnolli et al., *Cellular Immunology* 133:327-341, 1991; Bertagnolli, et al., *J.*  
20 *Immunol.* 149:3778-3783, 1992; Bowman et al., *J. Immunol.* 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: *Polyclonal T cell stimulation*, Kruisbeek, A.M. and Shevach, E.M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons,  
25 Toronto. 1994; and *Measurement of mouse and human Interferon  $\gamma$* , Schreiber, R.D. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: *Measurement of Human and Murine Interleukin 2 and Interleukin 4*, Bottomly, K., Davis, L.S. and  
30 Lipsky, P.E. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., *J. Exp. Med.* 173:1205-1211, 1991; Moreau et al., *Nature* 336:690-692, 1988; Greenberger et al., *Proc. Natl. Acad. Sci. U.S.A.* 80:2931-2938, 1983; *Measurement of mouse and human interleukin 6* - Nordan, R. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds.  
35 Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., *Proc. Natl.*



- 5 Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark, S.C. and Turner, K.J. In *Current Protocols in Immunology*. J.E.e.a. Coligan  
10 eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: *Current Protocols in Immunology*, Ed by J. E. Coligan, A.M. Kruisbeek,  
15 D.H. Margulies, E.M. Shevach, W Strober  
Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai  
20 et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

### **IMMUNE STIMULATING/SUPPRESSING ACTIVITY**

A protein of the present invention may also exhibit immune stimulating or  
25 immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell  
30 populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, leishmania, malaria  
35 and various fungal infections such as candida. Of course, in this regard, a protein of

5 the present invention may also be useful where a boost to the immune system generally would be indicated, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome,  
10 autoimmune thyroiditis, insulin dependent diabetes mellitus, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, asthma and related respiratory  
15 conditions), may also be treatable using a protein of the present invention.

A protein of the present invention may also suppress chronic or acute inflammation, such as, for example, that associated with infection (such as septic shock or systemic inflammatory response syndrome (SIRS)), inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF  
20 or IL-1 (such as the effect demonstrated by IL-11).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing  
25 Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al.,  
30 J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowman et al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular  
35 Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

5           Assays for T-cell-dependent immunoglobulin responses and isotype switching  
(which will identify, among others, proteins that modulate T-cell dependent antibody  
responses and that affect Th1/Th2 profiles) include, without limitation, those  
described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell  
function: *In vitro* antibody production, Mond, J.J. and Brunswick, M. In *Current*  
10 *Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and  
Sons, Toronto. 1994.

          Mixed lymphocyte reaction (MLR) assays (which will identify, among others,  
proteins that generate predominantly Th1 and CTL responses) include, without  
limitation, those described in: *Current Protocols in Immunology*, Ed by J. E. Coligan,  
15 A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing  
Associates and Wiley-Interscience (Chapter 3, *In Vitro* assays for Mouse Lymphocyte  
Function 3.1-3.19; Chapter 7, *Immunologic studies in Humans*); Takai et al., J.  
Immunol. 137:3494-3500, 1986; Takai et al.; J. Immunol. 140:508-512, 1988;  
Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

20           Dendritic cell-dependent assays (which will identify, among others, proteins  
expressed by denritic cells that activate naive T-cells) include, without limitation,  
those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., *Journal*  
*of Experimenal Medicine* 173:549-559, 1991; Macatonia et al., *Journal of*  
*Immunology* 154:5071-5079, 1995; Porgador et al., *Journal of Experimental Medicine*  
25 182:255-260, 1995; Nair et al., *Journal of Virology* 67:4062-4069, 1993; Huang et  
al., *Science* 264:961-965, 1994; Macatonia et al., *Journal of Experimental Medicine*  
169:1255-1264, 1989; Bhardwaj et al., *Journal of Clinical Investigation* 94:797-807,  
1994; and Inaba et al., *Journal of Experimental Medicine* 172:631-640, 1990.

          Assays for lymphocyte survival/apoptosis (which will identify, among others,  
30 proteins that prevent apoptosis after superantigen induction and proteins that regulate  
lymphocyte homeostasis) include, without limitation, those described in:  
Darzynkiewicz et al., *Cytometry* 13:795-808, 1992; Gorczyca et al., *Leukemia*  
7:659-670, 1993; Gorczyca et al., *Cancer Research* 53:1945-1951, 1993; Itoh et al.,  
*Cell* 66:233-243, 1991; Zacharchuk, *Journal of Immunology* 145:4037-4045, 1990;  
35 Zamai et al., *Cytometry* 14:891-897, 1993; Gorczyca et al., *International Journal of*  
*Oncology* 1:639-648, 1992.

5           Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

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### HEMATOPOIESIS REGULATING ACTIVITY

          A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent  
15   cell lines indicates involvement in regulating hematopoiesis. e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation  
20   of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use  
25   in place of or complementarily to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post  
30   irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (i.e. in conjunction with bone marrow transplantation) as normal cells or genetically manipulated for gene therapy.

          The activity of a protein of the invention may, among other means, be measured by the following methods:

35           Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

- 5            Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. *Cellular Biology* 15:141-151, 1995; Keller et al., *Molecular and Cellular Biology* 13:473-486, 1993; McClanahan et al., *Blood* 81:2903-2915, 1993.
- 10           Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M.G. In *Culture of Hematopoietic Cells*. R.I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., *Proc. Natl. Acad. Sci. USA* 89:5907-5911,
- 15           1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K. and Briddell, R.A. In *Culture of Hematopoietic Cells*. R.I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., *Experimental Hematology* 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In *Culture of Hematopoietic Cells*. R.I. Freshney, et al. eds. Vol pp.
- 20           1-21, Wiley-Liss, Inc., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In *Culture of Hematopoietic Cells*. R.I. Freshney, et al. eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initiating cell assay, Sutherland, H.J. In *Culture of Hematopoietic Cells*. R.I. Freshney, et al. eds. Vol pp. 139-162,
- 25           Wiley-Liss, Inc., New York, NY. 1994.

#### **TISSUE GENERATION/REGENERATION ACTIVITY**

A protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as

30           well as for wound healing and tissue repair, and in the treatment of burns, incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a

35           preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints.

5     *De novo* bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

10     A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

15     Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. *De novo* tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

5           The protein of the present invention may also be useful for proliferation of  
neural cells and for regeneration of nerve and brain tissue, *i.e.* for the treatment of  
central and peripheral nervous system diseases and neuropathies, as well as  
mechanical and traumatic disorders, which involve degeneration, death or trauma to  
neural cells or nerve tissue. More specifically, a protein may be used in the treatment  
10 of diseases of the peripheral nervous system, such as peripheral nerve injuries,  
peripheral neuropathy and localized neuropathies, and central nervous system  
diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic  
lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated  
in accordance with the present invention include mechanical and traumatic disorders,  
15 such as spinal cord disorders, head trauma and cerebrovascular diseases such as  
stroke. Peripheral neuropathies resulting from chemotherapy or other medical  
therapies may also be treatable using a protein of the invention.

It is expected that a protein of the present invention may also exhibit activity  
for generation of other tissues, such as organs (including, for example, pancreas, liver,  
20 intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and  
vascular (including vascular endothelium) tissue, or for promoting the growth of cells  
comprising such tissues. Part of the desired effects may be by inhibition of fibrotic  
scarring to allow normal tissue to regenerate.

A protein of the present invention may also be useful for gut protection or  
regeneration and treatment of lung or liver fibrosis, reperfusion injury in various  
25 tissues, and conditions resulting from systemic cytokine damage.

The activity of a protein of the invention may, among other means, be  
measured by the following methods:

Assays for tissue generation activity include, without limitation, those  
30 described in: International Patent Publication No. WO95/16035 (bone, cartilage,  
tendon); International Patent Publication No. WO95/05846 (nerve, neuronal);  
International Patent Publication No. WO91/07491 (skin, endothelium ).

#### **ACTIVIN/INHIBIN ACTIVITY**

35           A protein of the present invention may also exhibit activin- or inhibin-related  
activities. Inhibins are characterized by their ability to inhibit the release of follicle

5 stimulating hormone (FSH), while activins and are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in heterodimers with a member of the inhibin  $\alpha$  family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration  
10 of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin- $\beta$  group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, United States Patent 4,798,885.  
15 A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among other means, be measured by the following methods:

20 Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et al., Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci. USA 83:3091-3095, 1986.

#### 25 CHEMOTACTIC/CHEMOKINETIC ACTIVITY

A protein of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes, neutrophils, T-cells, mast cells, eosinophils and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilized or attract a desired  
30 cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

35 A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such



5 cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

10 The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for  
15 movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25: 1744-1748; Gruber et al. J. of Immunol. 152:5860-5867,  
20 1994; Johnston et al. J. of Immunol. 153: 1762-1768, 1994.

### **HEMOSTATIC AND THROMBOLYTIC ACTIVITY**

A protein of the invention may also exhibit hemostatic or thrombolytic  
25 activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of  
30 conditions resulting therefrom (such as, for example, infarction or stroke).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al.,  
35 Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

5

**RECEPTOR/LIGAND ACTIVITY**

A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

20

Suitable assays for receptor-ligand activity include without limitation those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltzenberg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

25

**OTHER ACTIVITIES**

30

A protein of the invention may also exhibit one or more of the following additional activities or effects: killing infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin or other tissue pigmentation, or organ size (such as, for example, breast augmentation or diminution); effecting the processing of dietary fat, protein or carbohydrate; effecting behavioral characteristics, including, without limitation, appetite, libido,

35

5 stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; and in the case of enzymes, correcting deficiencies of the enzyme and treating related diseases.

10

### **ADMINISTRATION AND DOSING**

A protein of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources) may be used in a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may also contain (in addition to protein and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin. The pharmaceutical composition may further contain other agents which either enhance the activity of the protein or complement its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein of the invention, or to minimize side effects. Conversely, protein of the present invention may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical compositions of the invention may comprise a protein of the invention in such multimeric or complexed form.

5           The pharmaceutical composition of the invention may be in the form of a complex of the protein(s) of present invention along with protein or peptide antigens. The protein and/or peptide antigen will deliver a stimulatory signal to both B and T lymphocytes. B lymphocytes will respond to antigen through their surface immunoglobulin receptor. T lymphocytes will respond to antigen through the T cell  
10 receptor (TCR) following presentation of the antigen by MHC proteins. MHC and structurally related proteins including those encoded by class I and class II MHC genes on host cells will serve to present the peptide antigen(s) to T lymphocytes. The antigen components could also be supplied as purified MHC-peptide complexes alone or with co-stimulatory molecules that can directly signal T cells. Alternatively  
15 antibodies able to bind surface immunoglobulin and other molecules on B cells as well as antibodies able to bind the TCR and other molecules on T cells can be combined with the pharmaceutical composition of the invention.

          The pharmaceutical composition of the invention may be in the form of a liposome in which protein of the present invention is combined, in addition to other  
20 pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within  
25 the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

          As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that  
30 is sufficient to show a meaningful patient benefit, i.e., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active  
35 ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

5 In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein of the present invention is administered to a mammal having a condition to be treated. Protein of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more  
10 cytokines, lymphokines or other hematopoietic factors, protein of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on  
15 the appropriate sequence of administering protein of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of protein of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in  
20 a variety of conventional ways, such as oral ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of protein of the present invention is administered orally, protein of the present invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the  
25 pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein of the present invention, and preferably from about 25 to 90% protein of the present invention. When administered in liquid form, a liquid carrier  
30 such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical  
35 composition contains from about 0.5 to 90% by weight of protein of the present invention, and preferably from about 1 to 50% protein of the present invention.

5           When a therapeutically effective amount of protein of the present invention is administered by intravenous, cutaneous or subcutaneous injection, protein of the present invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art.

10       A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may

15       also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

          The amount of protein of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has

20       undergone. Ultimately, the attending physician will decide the amount of protein of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of protein of the present invention and observe the patient's response. Larger doses of protein of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at

25       that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.01  $\mu$ g to about 100 mg (preferably about 0.1  $\mu$ g to about 10 mg, more preferably about 0.1  $\mu$ g to about 1 mg) of protein of the present invention per kg body weight.

30       The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the protein of the present invention will be in the range of 12 to 24 hours of continuous intravenous

35       administration. Ultimately the attending physician will decide on the appropriate

5 duration of intravenous therapy using the pharmaceutical composition of the present invention.

Protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the protein. Such antibodies may be obtained using either the entire protein or fragments thereof as an  
10 immunogen. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J. Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, *et al.*, FEBS Lett. 211, 10 (1987). Monoclonal antibodies binding to the  
15 protein of the invention may be useful diagnostic agents for the immunodetection of the protein. Neutralizing monoclonal antibodies binding to the protein may also be useful therapeutics for both conditions associated with the protein and also in the treatment of some forms of cancer where abnormal expression of the protein is involved. In the case of cancerous cells or leukemic cells, neutralizing monoclonal  
20 antibodies against the protein may be useful in detecting and preventing the metastatic spread of the cancerous cells, which may be mediated by the protein.

For compositions of the present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When  
25 administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein of the invention  
30 which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing composition to the site of bone and/or cartilage damage, providing a  
35 structure for the developing bone and cartilage and optimally capable of being

5 resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate formulation. Potential  
10 matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are  
15 nonbiodegradable and chemically defined, such as sintered hydroxapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size,  
20 particle shape, and biodegradability.

Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the protein  
25 compositions from disassociating from the matrix.

A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-  
30 methylcellulose, and carboxymethylcellulose, the most preferred being cationic salts of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt%, preferably 1-10 wt% based on total formulation weight, which represents the amount necessary to prevent desorption of the protein from the  
35 polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby



5 providing the protein the opportunity to assist the osteogenic activity of the progenitor cells.

In further compositions, proteins of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF- $\alpha$  and TGF- $\beta$ ), and insulin-like growth factor (IGF).  
10

The therapeutic compositions are also presently valuable for veterinary applications. Particularly domestic animals and thoroughbred horses, in addition to humans, are desired patients for such treatment with proteins of the present invention.

15 The dosage regimen of a protein-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the proteins, e.g., amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue (e.g., bone), the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage  
20 may vary with the type of matrix used in the reconstitution and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of  
25 tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced either *in vivo* or *ex vivo* into cells for expression in a mammalian subject. Polynucleotides of the invention may also be  
30 administered by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA).

Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes.

35

5           Patent and literature references cited herein are incorporated by reference as  
if fully set forth.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Jacobs, Kenneth  
McCoy, John  
Kelleher, Kerry  
Carlin, McKeough

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAGAAGATAA AACTGGACAC TGGGGAGACA CAACTTC ATG CTG CGT GGG ATC TCC	55
Met Leu Arg Gly Ile Ser	
1 5	
CAG CTA CCT GCA GTG GCC ACC ATG TCT TGG GTC CTG CTG CCT GTA CTT	103
Gln Leu Pro Ala Val Ala Thr Met Ser Trp Val Leu Leu Pro Val Leu	
10 15 20	
TGG CTC ATT GTT CAA ACT CAA GCA ATA GCC ATA AAG CAA ACA CCT GAA	151
Trp Leu Ile Val Gln Thr Gln Ala Ile Ala Ile Lys Gln Thr Pro Glu	
25 30 35	
TTA ACG CTC CAT GAA ATA GTT TGT CCT AAA AAA CTT CAC ATT TTA CAC	199
Leu Thr Leu His Glu Ile Val Cys Pro Lys Lys Leu His Ile Leu His	
40 45 50	
AAA AGA GAG ATC AAG AAC AAC CAG ACA GAA AAG CAT GGC AAA GAG GAA	247
Lys Arg Glu Ile Lys Asn Asn Gln Thr Glu Lys His Gly Lys Glu Glu	
55 60 65 70	
AGG TAT GAA CCT GAA GTT CAA TAT CAG ATG ATC TTA AAT GGA GAA GAA	295
Arg Tyr Glu Pro Glu Val Gln Tyr Gln Met Ile Leu Asn Gly Glu Glu	
75 80 85	
ATC ATT CTC TCC CTA CAA AAA ACC AAG CAC CTC CTG GGG CCA GAC TAC	343
Ile Ile Leu Ser Leu Gln Lys Thr Lys His Leu Leu Gly Pro Asp Tyr	
90 95 100	
ACT GAA ACA TTG TAC TCA CCC AGA GGA GAG GAA ATT ACC ACG AAA CCT	391
Thr Glu Thr Leu Tyr Ser Pro Arg Gly Glu Glu Ile Thr Thr Lys Pro	
105 110 115	
GAG AAC ATG GAA CAC TGT TAC TAT AAA GGA AAC ATC CTA AAT GAA AAG	439
Glu Asn Met Glu His Cys Tyr Tyr Lys Gly Asn Ile Leu Asn Glu Lys	
120 125 130	
AAT TCT GTT GCC AGC ATC AGT ACT TGT GAC GGG TTG AGA GGA TAC TTC	487
Asn Ser Val Ala Ser Ile Ser Thr Cys Asp Gly Leu Arg Gly Tyr Phe	
135 140 145 150	
ACA CAT CAT CAC CAA AGA TAC CAG ATA AAA CCT CTG AAA AGC ACA GAC	535
Thr His His His Gln Arg Tyr Gln Ile Lys Pro Leu Lys Ser Thr Asp	
155 160 165	
GAG AAA GAA CAT GCC GTC TTT ACA TCT AAC CAG GAG GAA CAA GAC CCA	583
Glu Lys Glu His Ala Val Phe Thr Ser Asn Gln Glu Glu Gln Asp Pro	
170 175 180	
GCT AAC CAC ACA TGT GGT GTG AAG AGC ACT GAC GGG AAA CAA GGC CCA	631
Ala Asn His Thr Cys Gly Val Lys Ser Thr Asp Gly Lys Gln Gly Pro	
185 190 195	
ATT CGA ATC TCT AGA TCA CTC AAA AGC CCA GAG AAA GAA GAC TTT CTT	679
Ile Arg Ile Ser Arg Ser Leu Lys Ser Pro Glu Lys Glu Asp Phe Leu	
200 205 210	
CGG GCA CAG AAA TAC ATT GAT CTC TAT TTG GTG CTG GAT AAT GCC TTT	727
Arg Ala Gln Lys Tyr Ile Asp Leu Tyr Leu Val Leu Asp Asn Ala Phe	
215 220 225 230	
TAT AAG AAC TAT AAT GAG AAT CTA ACT CTG ATA AGA AGC TTT GTG TTT	775

Tyr	Lys	Asn	Tyr	Asn	Glu	Asn	Leu	Thr	Leu	Ile	Arg	Ser	Phe	Val	Phe
				235					240					245	

GAT GTG ATG AAC CTA CTC AAT GTG ATA TAT AAC ACC ATA GAT GTT CAA Asp Val Met Asn Leu Leu Asn Val Ile Tyr Asn Thr Ile Asp Val Gln 250 255 260	823
GTG GCC TTG GTA GGT ATG GAA ATC TGG TCT GAT GGG GAT AAG ATA AAG Val Ala Leu Val Gly Met Glu Ile Trp Ser Asp Gly Asp Lys Ile Lys 265 270 275	871
GTG GTG CCC AGC GCA AGC ACC ACG TTT GAC AAC TTC CTG AGA TGG CAC Val Val Pro Ser Ala Ser Thr Thr Phe Asp Asn Phe Leu Arg Trp His 280 285 290	919
AGT TCT AAC CTG GGG AAA AAG ATC CAC GAC CAT GCT CAG CTT CTC AGC Ser Ser Asn Leu Gly Lys Lys Ile His Asp His Ala Gln Leu Leu Ser 295 300 305 310	967
GGG ATT AGC TTC AAC AAT CGA CGT GTG GGA CTG GCA GCT TCA AAT TCC Gly Ile Ser Phe Asn Asn Arg Arg Val Gly Leu Ala Ala Ser Asn Ser 315 320 325	1015
TTG TGT TCC CCA TCT TCG GTT GCT GTT ATT GAG GCT AAA AAA AAG AAT Leu Cys Ser Pro Ser Ser Val Ala Val Ile Glu Ala Lys Lys Lys Asn 330 335 340	1063
AAT GTG GCT CTT GTA GGA GTG ATG TCA CAT GAG CTG GGC CAT GTC CTT Asn Val Ala Leu Val Gly Val Met Ser His Glu Leu Gly His Val Leu 345 350 355	1111
GGT ATG CCT GAT GTT CCA TTC AAC ACC AAG TGT CCC TCT GGC AGT TGT Gly Met Pro Asp Val Pro Phe Asn Thr Lys Cys Pro Ser Gly Ser Cys 360 365 370	1159
GTG ATG AAT CAG TAT CTG AGT TCA AAA TTC CCA AAG GAT TTC AGT ACA Val Met Asn Gln Tyr Leu Ser Ser Lys Phe Pro Lys Asp Phe Ser Thr 375 380 385 390	1207
TCT TGC CGT GCA CAT TTT GAA AGA TAC CTT TTA TCT CAG AAA CCA AAG Ser Cys Arg Ala His Phe Glu Arg Tyr Leu Leu Ser Gln Lys Pro Lys 395 400 405	1255
TGC CTG CTG CAA GCA CCT ATT CCT ACA AAT ATA ATG ACA ACA CCA GTG Cys Leu Leu Gln Ala Pro Ile Pro Thr Asn Ile Met Thr Thr Pro Val 410 415 420	1303
TGT GGG AAC CAC CTT CTA GAA GTG GGA GAA GAC TGT GAT TGT GGC TCT Cys Gly Asn His Leu Leu Glu Val Gly Glu Asp Cys Asp Cys Gly Ser 425 430 435	1351
CCT AAG GAG TGT ACC AAT CTC TGC TGT GAA GCC CTA ACG TGT AAA CTG Pro Lys Glu Cys Thr Asn Leu Cys Cys Glu Ala Leu Thr Cys Lys Leu 440 445 450	1399
AAG CCT GGA ACT GAT TGC GGA GGA GAT GCT CCA AAC CAT ACC ACA GAG Lys Pro Gly Thr Asp Cys Gly Gly Asp Ala Pro Asn His Thr Thr Glu 455 460 465 470	1447
TGAATCCAAA AGTCTGCTTC ACTGAGATGC TACCTTGCCA GGACAAGAAC CAAGAACTCT	1507
AACTGTCCCA GGAATCTTGT GAATTTTCAC CCATAATGGT CTTTCACTTG TCATTCTACT	1567
TTCTATATTG TTATCAGTCC AGGAAACAGG TAAACAGATG TAATTAGAGA CATTGGCTCT	1627
TTGTTTAGGC CTAATCTTTC TTTTACTTTT TTTTTCCTTT TTTTCTTTTT TTTTAAAGAT	1687

CATGAATTTG TGACTTAGTT CTGCCCTTTG GAGAACAAAA GAAAGCAGTC TTCCATCAAA 1747  
 TCACCTTAAA ATGCACGGCT AAACATTCA GAGTTAACAC TCCAGAATTG TTAAATTACA 1807  
 AGTACTATGC TTTAATGCTT CTTTCATCTT ACTAGTATGG CCTATAAAAA AAATAATACC 1867  
 ACTTGATGGG TGAAGGCTTT GGCAATAGAA AGAAGAATAG AATTCAGGTT TTATGTTATT 1927  
 CCTCTGTGTT CACTTCGCCT TGCTCTTGAA AGTGCAGTAT TTTTCTACAT CATGTCGAGA 1987  
 ATGATTCAAT GTAAATATTT TTCATTTTAT CATGTATATC CTATACACAC ATCTCCTTCA 2047  
 TCATCATATA TGAAGTTTAT TTTGAGAAGT CTACATTGCT TACATTTTAA TTGAGCCAGC 2107  
 AAAGAAGGCT TAATGATTTA TTGAACCATA ATGTCAATAA AAACACAAC TTTGAGGCAA 2167  
 AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AA 2209

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 470 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Leu Arg Gly Ile Ser Gln Leu Pro Ala Val Ala Thr Met Ser Trp  
 1 5 10 15  
 Val Leu Leu Pro Val Leu Trp Leu Ile Val Gln Thr Gln Ala Ile Ala  
 20 25 30  
 Ile Lys Gln Thr Pro Glu Leu Thr Leu His Glu Ile Val Cys Pro Lys  
 35 40 45  
 Lys Leu His Ile Leu His Lys Arg Glu Ile Lys Asn Asn Gln Thr Glu  
 50 55 60  
 Lys His Gly Lys Glu Glu Arg Tyr Glu Pro Glu Val Gln Tyr Gln Met  
 65 70 75 80  
 Ile Leu Asn Gly Glu Glu Ile Ile Leu Ser Leu Gln Lys Thr Lys His  
 85 90 95  
 Leu Leu Gly Pro Asp Tyr Thr Glu Thr Leu Tyr Ser Pro Arg Gly Glu  
 100 105 110  
 Glu Ile Thr Thr Lys Pro Glu Asn Met Glu His Cys Tyr Tyr Lys Gly  
 115 120 125  
 Asn Ile Leu Asn Glu Lys Asn Ser Val Ala Ser Ile Ser Thr Cys Asp  
 130 135 140  
 Gly Leu Arg Gly Tyr Phe Thr His His His Gln Arg Tyr Gln Ile Lys  
 145 150 155 160  
 Pro Leu Lys Ser Thr Asp Glu Lys Glu His Ala Val Phe Thr Ser Asn  
 165 170 175

Gln Glu Glu Gln Asp Pro Ala Asn His Thr Cys Gly Val Lys Ser Thr  
 180 185 190  
 Asp Gly Lys Gln Gly Pro Ile Arg Ile Ser Arg Ser Leu Lys Ser Pro  
 195 200 205  
 Glu Lys Glu Asp Phe Leu Arg Ala Gln Lys Tyr Ile Asp Leu Tyr Leu  
 210 215 220  
 Val Leu Asp Asn Ala Phe Tyr Lys Asn Tyr Asn Glu Asn Leu Thr Leu  
 225 230 235 240  
 Ile Arg Ser Phe Val Phe Asp Val Met Asn Leu Leu Asn Val Ile Tyr  
 245 250 255  
 Asn Thr Ile Asp Val Gln Val Ala Leu Val Gly Met Glu Ile Trp Ser  
 260 265 270  
 Asp Gly Asp Lys Ile Lys Val Val Pro Ser Ala Ser Thr Thr Phe Asp  
 275 280 285  
 Asn Phe Leu Arg Trp His Ser Ser Asn Leu Gly Lys Lys Ile His Asp  
 290 295 300  
 His Ala Gln Leu Leu Ser Gly Ile Ser Phe Asn Asn Arg Arg Val Gly  
 305 310 315 320  
 Leu Ala Ala Ser Asn Ser Leu Cys Ser Pro Ser Ser Val Ala Val Ile  
 325 330 335  
 Glu Ala Lys Lys Lys Asn Asn Val Ala Leu Val Gly Val Met Ser His  
 340 345 350  
 Glu Leu Gly His Val Leu Gly Met Pro Asp Val Pro Phe Asn Thr Lys  
 355 360 365  
 Cys Pro Ser Gly Ser Cys Val Met Asn Gln Tyr Leu Ser Ser Lys Phe  
 370 375 380  
 Pro Lys Asp Phe Ser Thr Ser Cys Arg Ala His Phe Glu Arg Tyr Leu  
 385 390 395 400  
 Leu Ser Gln Lys Pro Lys Cys Leu Leu Gln Ala Pro Ile Pro Thr Asn  
 405 410 415  
 Ile Met Thr Thr Pro Val Cys Gly Asn His Leu Leu Glu Val Gly Glu  
 420 425 430  
 Asp Cys Asp Cys Gly Ser Pro Lys Glu Cys Thr Asn Leu Cys Cys Glu  
 435 440 445  
 Ala Leu Thr Cys Lys Leu Lys Pro Gly Thr Asp Cys Gly Gly Asp Ala  
 450 455 460  
 Pro Asn His Thr Thr Glu  
 465 470

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2582 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double



(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 52..2034

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATTTCTCAGC	TCCAAGCATT	AGGTAAACCC	ACCAAGCAAT	CCTAGCCTGT	G	ATG	GCG		57							
							Met	Ala								
							1									
TTT	GAC	GTC	AGC	TGC	TTC	TTT	TGG	GTG	GTG	CTG	TTT	TCT	GCC	GCG	TGT	105
Phe	Asp	Val	Ser	Cys	Phe	Phe	Trp	Val	Val	Leu	Phe	Ser	Ala	Gly	Cys	
		5					10					15				
AAA	GTC	ATC	ACC	TCC	TGG	GAT	CAG	ATG	TGC	ATT	GAG	AAA	GAA	GCC	AAC	153
Lys	Val	Ile	Thr	Ser	Trp	Asp	Gln	Met	Cys	Ile	Glu	Lys	Glu	Ala	Asn	
	20					25					30					
AAA	ACA	TAT	AAC	TGT	GAA	AAT	TTA	GGT	CTC	AGT	GAA	ATC	CCT	GAC	ACT	201
Lys	Thr	Tyr	Asn	Cys	Glu	Asn	Leu	Gly	Leu	Ser	Glu	Ile	Pro	Asp	Thr	
	35				40				45					50		
CTA	CCA	AAC	ACA	ACA	GAA	TTT	TTG	GAA	TTC	AGC	TTT	AAT	TTT	TTG	CCT	249
Leu	Pro	Asn	Thr	Thr	Glu	Phe	Leu	Glu	Phe	Ser	Phe	Asn	Phe	Leu	Pro	
				55				60						65		
ACA	ATT	CAC	AAT	AGA	ACC	TTC	AGC	AGA	CTC	ATG	AAT	CTT	ACC	TTT	TTG	297
Thr	Ile	His	Asn	Arg	Thr	Phe	Ser	Arg	Leu	Met	Asn	Leu	Thr	Phe	Leu	
			70					75					80			
GAT	TTA	ACT	AGG	TGC	CAG	ATT	AAC	TGG	ATA	CAT	GAA	GAC	ACT	TTT	CAA	345
Asp	Leu	Thr	Arg	Cys	Gln	Ile	Asn	Trp	Ile	His	Glu	Asp	Thr	Phe	Gln	
		85				90						95				
AGC	CAT	CAT	CAA	TTA	AGC	ACA	CTT	GTG	TTA	ACT	GGA	AAT	CCC	CTG	ATA	393
Ser	His	His	Gln	Leu	Ser	Leu	Leu	Val	Leu	Thr	Gly	Asn	Pro	Leu	Ile	
	100					105					110					
TTC	ATG	GCA	GAA	ACA	TCG	CTT	AAT	GGG	CCC	AAG	TCA	CTG	AAG	CAT	CTT	441
Phe	Met	Ala	Glu	Thr	Ser	Leu	Asn	Gly	Pro	Lys	Ser	Leu	Lys	His	Leu	
	115				120				125					130		
TTC	TTA	ATC	CAA	ACG	GGA	ATA	TCC	AAT	CTC	GAG	TTT	ATT	CCA	GTG	CAC	489
Phe	Leu	Ile	Gln	Thr	Gly	Ile	Ser	Asn	Leu	Glu	Phe	Ile	Pro	Val	His	
			135						140					145		
AAT	CTG	GAA	AAC	TTG	GAA	AGC	TTG	TAT	CTT	GGA	AGC	AAC	CAT	ATT	TCC	537
Asn	Leu	Glu	Asn	Leu	Glu	Ser	Leu	Tyr	Leu	Gly	Ser	Asn	His	Ile	Ser	
		150						155					160			
TCC	ATT	AAG	TTC	CCC	AAA	GAC	TTC	CCA	GCA	CGG	AAT	CTG	AAA	GTA	CTG	585
Ser	Ile	Lys	Phe	Pro	Lys	Asp	Phe	Pro	Ala	Arg	Asn	Leu	Lys	Val	Leu	
		165					170					175				
GAT	TTT	CAG	AAT	AAT	GCT	ATA	CAC	TAC	ATC	TCT	AGA	GAA	GAC	ATG	AGG	633

Asp	Phe	Gln	Asn	Asn	Ala	Ile	His	Tyr	Ile	Ser	Arg	Glu	Asp	Met	Arg	
180						185					190					
TCT	CTG	GAG	CAG	GCC	ATC	AAC	CTA	AGC	CTG	AAC	TTC	AAT	GGC	AAT	AAT	681
Ser	Leu	Glu	Gln	Ala	Ile	Asn	Leu	Ser	Leu	Asn	Phe	Asn	Gly	Asn	Asn	
195				200						205					210	
GTT	AAA	GGT	ATT	GAG	CTT	GGG	GCT	TTT	GAT	TCA	ACG	GTC	TTC	CAA	AGT	729
Val	Lys	Gly	Ile	Glu	Leu	Gly	Ala	Phe	Asp	Ser	Thr	Val	Phe	Gln	Ser	
			215						220					225		
TTG	AAC	TTT	GGA	GGA	ACT	CCA	AAT	TTG	TCT	GTT	ATA	TTC	AAT	GGT	CTG	777
Leu	Asn	Phe	Gly	Gly	Thr	Pro	Asn	Leu	Ser	Val	Ile	Phe	Asn	Gly	Leu	
			230					235					240			
CAG	AAC	TCT	ACT	ACT	CAG	TCT	CTC	TGG	CTG	GGA	ACA	TTT	GAG	GAC	ATT	825
Gln	Asn	Ser	Thr	Thr	Gln	Ser	Leu	Trp	Leu	Gly	Thr	Phe	Glu	Asp	Ile	
		245					250					255				
GAT	GAC	GAA	GAT	ATT	AGT	TCA	GCC	ATG	CTC	AAG	GGA	CTC	TGT	GAA	ATG	873
Asp	Asp	Glu	Asp	Ile	Ser	Ser	Ala	Met	Leu	Lys	Gly	Leu	Cys	Glu	Met	
	260					265					270					
TCT	GTT	GAG	AGC	CTC	AAC	CTG	CAG	GAA	CAC	CGC	TTC	TCT	GAC	ATC	TCA	921
Ser	Val	Glu	Ser	Leu	Asn	Leu	Gln	Glu	His	Arg	Phe	Ser	Asp	Ile	Ser	
275					280					285					290	
TCC	ACC	ACA	TTT	CAG	TGC	TTC	ACC	CAA	CTC	CAA	GAA	TTG	GAT	CTG	ACA	969
Ser	Thr	Thr	Phe	Gln	Cys	Phe	Thr	Gln	Leu	Gln	Glu	Leu	Asp	Leu	Thr	
			295					300						305		
GCA	ACT	CAC	TTG	AAA	GGG	TTA	CCC	TCT	GGG	ATG	AAG	GGT	CTG	AAC	TTG	1017
Ala	Thr	His	Leu	Lys	Gly	Leu	Pro	Ser	Gly	Met	Lys	Gly	Leu	Asn	Leu	
			310					315					320			
CTC	AAG	AAA	TTA	GTT	CTC	AGT	GTA	AAT	CAT	TTC	GAT	CAA	TTG	TGT	CAA	1065
Leu	Lys	Lys	Leu	Val	Leu	Ser	Val	Asn	His	Phe	Asp	Gln	Leu	Cys	Gln	
		325					330					335				
ATC	AGT	GCT	GCC	AAT	TTC	CCC	TCC	CTT	ACA	CAC	CTC	TAC	ATC	AGA	GGC	1113
Ile	Ser	Ala	Ala	Asn	Phe	Pro	Ser	Leu	Thr	His	Leu	Tyr	Ile	Arg	Gly	
	340					345					350					
AAC	GTG	AAG	AAA	CTT	CAC	CTT	GGT	GTT	GGC	TGC	TTG	GAG	AAA	CTA	GGA	1161
Asn	Val	Lys	Lys	Leu	His	Leu	Gly	Val	Gly	Cys	Leu	Glu	Lys	Leu	Gly	
355					360				365						370	
AAC	CTT	CAG	ACA	CTT	GAT	TTA	AGC	CAT	AAT	GAC	ATA	GAG	GCT	TCT	GAC	1209
Asn	Leu	Gln	Thr	Leu	Asp	Leu	Ser	His	Asn	Asp	Ile	Glu	Ala	Ser	Asp	
			375					380						385		
TGC	TGC	AGT	CTG	CAA	CTC	AAA	AAC	CTG	TCC	CAC	TTG	CAA	ACC	TTA	AAC	1257
Cys	Cys	Ser	Leu	Gln	Leu	Lys	Asn	Leu	Ser	His	Leu	Gln	Thr	Leu	Asn	
			390					395					400			
CTG	AGC	CAC	AAT	GAG	CCT	CTT	GGT	CTC	CAG	AGT	CAG	GCA	TTC	AAA	GAA	1305
Leu	Ser	His	Asn	Glu	Pro	Leu	Gly	Leu	Gln	Ser	Gln	Ala	Phe	Lys	Glu	
		405					410					415				
TGT	CCT	CAG	CTA	GAA	CTC	CTC	GAT	TTG	GCA	TTT	ACC	CGC	TTA	CAC	ATT	1353
Cys	Pro	Gln	Leu	Glu	Leu	Leu	Asp	Leu	Ala	Phe	Thr	Arg	Leu	His	Ile	
	420					425					430					

AAT GCT CCA CAA AGT CCC TTC CAA AAC CTC CAT TTC CTT CAG GTT CTG Asn Ala Pro Gln Ser Pro Phe Gln Asn Leu His Phe Leu Gln Val Leu 435 440 445 450	1401
AAT CTC ACT TAC TGC TTC CTT GAT ACC AGC AAT CAG CAT CTT CTA GCA Asn Leu Thr Tyr Cys Phe Leu Asp Thr Ser Asn Gln His Leu Leu Ala 455 460 465	1449
GGC CTA CCA GTT CTC CGG CAT CTC AAC TTA AAA GGG AAT CAC TTT CAA Gly Leu Pro Val Leu Arg His Leu Asn Leu Lys Gly Asn His Phe Gln 470 475 480	1497
GAT GGG ACT ATC ACG AAG ACC AAC CTA CTT CAG ACC GTG GGC AGC TTG Asp Gly Thr Ile Thr Lys Thr Asn Leu Leu Gln Thr Val Gly Ser Leu 485 490 495	1545
GAG GTT CTG ATT TTG TCC TCT TGT GGT CTC CTC TCT ATA GAC CAG CAA Glu Val Leu Ile Leu Ser Ser Cys Gly Leu Leu Ser Ile Asp Gln Gln 500 505 510	1593
GCA TTC CAC AGC TTG GGA AAA ATG AGC CAT GTA GAC TTA AGC CAC AAC Ala Phe His Ser Leu Gly Lys Met Ser His Val Asp Leu Ser His Asn 515 520 525 530	1641
AGC CTG ACA TGC GAC AGC ATT GAT TCT CTT AGC CAT CTT AAG GGA ATC Ser Leu Thr Cys Asp Ser Ile Asp Ser Leu Ser His Leu Lys Gly Ile 535 540 545	1689
TAC CTC AAT CTG GCT GCC AAC AGC ATT AAC ATC ATC TCA CCC CGT CTC Tyr Leu Asn Leu Ala Ala Asn Ser Ile Asn Ile Ile Ser Pro Arg Leu 550 555 560	1737
CTC CCT ATC TTG TCC CAG CAG AGC ACC ATT AAT TTA AGT CAT AAC CCC Leu Pro Ile Leu Ser Gln Gln Ser Thr Ile Asn Leu Ser His Asn Pro 565 570 575	1785
CTG GAC TGC ACT TGC TCG AAT ATT CAT TTC TTA ACA TGG TAC AAA GAA Leu Asp Cys Thr Cys Ser Asn Ile His Phe Leu Thr Trp Tyr Lys Glu 580 585 590	1833
AAC CTG CAC AAA CTT GAA GGC TCG GAG GAG ACC ACG TGT GCA AAC CCG Asn Leu His Lys Leu Glu Gly Ser Glu Glu Thr Thr Cys Ala Asn Pro 595 600 605 610	1881
CCA TCT CTA AGG GGA GTT AAG CTA TCT GAT GTC AAG CTT TCC TGT GGG Pro Ser Leu Arg Gly Val Lys Leu Ser Asp Val Lys Leu Ser Cys Gly 615 620 625	1929
ATT ACA GCC ATA GGC ATT TTC TTT CTC ATA GTA TTT CTA TTA TTG TTG Ile Thr Ala Ile Gly Ile Phe Phe Leu Ile Val Phe Leu Leu Leu Leu 630 635 640	1977
GCT ATT CTG CTA TTT TTT GCA GTT AAA TAC CTT CTC AGG TGG AAA TAC Ala Ile Leu Leu Phe Phe Ala Val Lys Tyr Leu Leu Arg Trp Lys Tyr 645 650 655	2025
CAA CAC ATT TAGTGCTGAA GGTTCACAGA GAAAGCAAAT AAGTGTGCTT Gln His Ile 660	2074
AGCAAAATTG CTCTAAGTGA AAGAACTGTC ATCTGCTGGT GACCAGACCA GACTTTTCAG	2134
ATTGCTTCCT GGAAGTGGGC AGGGACTCAC TGTGCTTTTC TGAGCTTCCTT ACTCCTGTGA	2194

GTCCCAGAGC TAAAGAACCT TCTAGGCAAG TACACCGAAT GACTCAGTCC AGAGGGTCAG	2254
ATGCTGCTGT GAGAGGCACA GAGCCCTTTC CGCATGTGGA AGAGTGGGAG GAAGCAGAGG	2314
GAGGGACTGG GCAGGGACTG CCGGCCCGG AGTCTCCAC AGGGAGGCCA TTCCCCTTCT	2374
ACTCACCGAC ATCCCTCCCA GCACCACACA CCCC GCCCT GAAAGGAGAT CATCAGCCCC	2434
CACAATTTGT CAGAGCTGAA GCCAGCCAC TACCCACCCC CACTACAGCA TTGTGCTTGG	2494
GTCTGGGTTC TCAGTAATGT AGCCATTTGA GAACTTACT TGGGGACAAA GTCTCAATCC	2554
TTATTTTAAA TGAAAAAAAA AAAAAAA	2582

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 661 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Ala	Phe	Asp	Val	Ser	Cys	Phe	Phe	Trp	Val	Val	Leu	Phe	Ser	Ala	1	5	10	15
Gly	Cys	Lys	Val	Ile	Thr	Ser	Trp	Asp	Gln	Met	Cys	Ile	Glu	Lys	Glu	20	25	30	
Ala	Asn	Lys	Thr	Tyr	Asn	Cys	Glu	Asn	Leu	Gly	Leu	Ser	Glu	Ile	Pro	35	40	45	
Asp	Thr	Leu	Pro	Asn	Thr	Thr	Glu	Phe	Leu	Glu	Phe	Ser	Phe	Asn	Phe	50	55	60	
Leu	Pro	Thr	Ile	His	Asn	Arg	Thr	Phe	Ser	Arg	Leu	Met	Asn	Leu	Thr	65	70	75	80
Phe	Leu	Asp	Leu	Thr	Arg	Cys	Gln	Ile	Asn	Trp	Ile	His	Glu	Asp	Thr	85	90	95	
Phe	Gln	Ser	His	His	Gln	Leu	Ser	Thr	Leu	Val	Leu	Thr	Gly	Asn	Pro	100	105	110	
Leu	Ile	Phe	Met	Ala	Glu	Thr	Ser	Leu	Asn	Gly	Pro	Lys	Ser	Leu	Lys	115	120	125	
His	Leu	Phe	Leu	Ile	Gln	Thr	Gly	Ile	Ser	Asn	Leu	Glu	Phe	Ile	Pro	130	135	140	
Val	His	Asn	Leu	Glu	Asn	Leu	Glu	Ser	Leu	Tyr	Leu	Gly	Ser	Asn	His	145	150	155	160
Ile	Ser	Ser	Ile	Lys	Phe	Pro	Lys	Asp	Phe	Pro	Ala	Arg	Asn	Leu	Lys	165	170	175	
Val	Leu	Asp	Phe	Gln	Asn	Asn	Ala	Ile	His	Tyr	Ile	Ser	Arg	Glu	Asp	180	185	190	
Met	Arg	Ser	Leu	Glu	Gln	Ala	Ile	Asn	Leu	Ser	Leu	Asn	Phe	Asn	Gly				

195					200					205					
Asn	Asn	Val	Lys	Gly	Ile	Glu	Leu	Gly	Ala	Phe	Asp	Ser	Thr	Val	Phe
210						215					220				
Gln	Ser	Leu	Asn	Phe	Gly	Gly	Thr	Pro	Asn	Leu	Ser	Val	Ile	Phe	Asn
225					230					235					240
Gly	Leu	Gln	Asn	Ser	Thr	Thr	Gln	Ser	Leu	Trp	Leu	Gly	Thr	Phe	Glu
				245					250					255	
Asp	Ile	Asp	Asp	Glu	Asp	Ile	Ser	Ser	Ala	Met	Leu	Lys	Gly	Leu	Cys
			260					265					270		
Glu	Met	Ser	Val	Glu	Ser	Leu	Asn	Leu	Gln	Glu	His	Arg	Phe	Ser	Asp
		275					280					285			
Ile	Ser	Ser	Thr	Thr	Phe	Gln	Cys	Phe	Thr	Gln	Leu	Gln	Glu	Leu	Asp
	290					295					300				
Leu	Thr	Ala	Thr	His	Leu	Lys	Gly	Leu	Pro	Ser	Gly	Met	Lys	Gly	Leu
305					310					315					320
Asn	Leu	Leu	Lys	Lys	Leu	Val	Leu	Ser	Val	Asn	His	Phe	Asp	Gln	Leu
				325					330					335	
Cys	Gln	Ile	Ser	Ala	Ala	Asn	Phe	Pro	Ser	Leu	Thr	His	Leu	Tyr	Ile
			340					345					350		
Arg	Gly	Asn	Val	Lys	Lys	Leu	His	Leu	Gly	Val	Gly	Cys	Leu	Glu	Lys
		355					360					365			
Leu	Gly	Asn	Leu	Gln	Thr	Leu	Asp	Leu	Ser	His	Asn	Asp	Ile	Glu	Ala
	370					375					380				
Ser	Asp	Cys	Cys	Ser	Leu	Gln	Leu	Lys	Asn	Leu	Ser	His	Leu	Gln	Thr
385					390					395					400
Leu	Asn	Leu	Ser	His	Asn	Glu	Pro	Leu	Gly	Leu	Gln	Ser	Gln	Ala	Phe
				405					410					415	
Lys	Glu	Cys	Pro	Gln	Leu	Glu	Leu	Leu	Asp	Leu	Ala	Phe	Thr	Arg	Leu
			420					425					430		
His	Ile	Asn	Ala	Pro	Gln	Ser	Pro	Phe	Gln	Asn	Leu	His	Phe	Leu	Gln
		435					440					445			
Val	Leu	Asn	Leu	Thr	Tyr	Cys	Phe	Leu	Asp	Thr	Ser	Asn	Gln	His	Leu
	450					455					460				
Leu	Ala	Gly	Leu	Pro	Val	Leu	Arg	His	Leu	Asn	Leu	Lys	Gly	Asn	His
465					470					475					480
Phe	Gln	Asp	Gly	Thr	Ile	Thr	Lys	Thr	Asn	Leu	Leu	Gln	Thr	Val	Gly
				485					490					495	
Ser	Leu	Glu	Val	Leu	Ile	Leu	Ser	Ser	Cys	Gly	Leu	Leu	Ser	Ile	Asp
			500					505					510		
Gln	Gln	Ala	Phe	His	Ser	Leu	Gly	Lys	Met	Ser	His	Val	Asp	Leu	Ser
		515					520					525			
His	Asn	Ser	Leu	Thr	Cys	Asp	Ser	Ile	Asp	Ser	Leu	Ser	His	Leu	Lys

530	535	540
Gly Ile Tyr Leu Asn Leu Ala Ala Asn Ser Ile Asn Ile Ile Ser Pro		
545	550	555 560
Arg Leu Leu Pro Ile Leu Ser Gln Gln Ser Thr Ile Asn Leu Ser His		
	565	570 575
Asn Pro Leu Asp Cys Thr Cys Ser Asn Ile His Phe Leu Thr Trp Tyr		
	580	585 590
Lys Glu Asn Leu His Lys Leu Glu Gly Ser Glu Glu Thr Thr Cys Ala		
	595	600 605
Asn Pro Pro Ser Leu Arg Gly Val Lys Leu Ser Asp Val Lys Leu Ser		
	610	615 620
Cys Gly Ile Thr Ala Ile Gly Ile Phe Phe Leu Ile Val Phe Leu Leu		
	625	630 635 640
Leu Leu Ala Ile Leu Leu Phe Phe Ala Val Lys Tyr Leu Leu Arg Trp		
	645	650 655
Lys Tyr Gln His Ile		
	660	

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 588 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 76..474

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGGCCAAAGA GGCCTAAACT TCGGGCTGTC CATCTCACCT ACAGCTCTGG TCTCATCCTC	60
AACTCAACCA CAATC ATG GCT CAG ATG ATG ACT CTG AGC CTC CTT AGC CTG	111
Met Ala Gln Met Met Thr Leu Ser Leu Leu Ser Leu	
1 5 10	
GTC CTG GCT CTC TGC ATC CCC TGG ACC CAA GGC AGT GAT GGA GGG GGT	159
Val Leu Ala Leu Cys Ile Pro Trp Thr Gln Gly Ser Asp Gly Gly Gly	
15 20 25	
CAG GAC TGC TGC CTT AAG TAC AGC CAG AAG AAA ATT CCC TAC AGT ATT	207
Gln Asp Cys Cys Leu Lys Tyr Ser Gln Lys Lys Ile Pro Tyr Ser Ile	
30 35 40	
GTC CGA GGC TAT AGG AAG CAA GAA CCA AGT TTA GGC TGT CCC ATC CCG	255
Val Arg Gly Tyr Arg Lys Gln Glu Pro Ser Leu Gly Cys Pro Ile Pro	
45 50 55 60	

GCA ATC CTG TTC TCA CCC CGG AAG CAC TCT AAG CCT GAG CTA TGT GCA	303
Ala Ile Leu Phe Ser Pro Arg Lys His Ser Lys Pro Glu Leu Cys Ala	
65 70 75	
AAC CCT GAG GAA GGC TGG GTG CAG AAC CTG ATG CGC CGC CTG GAC CAG	351
Asn Pro Glu Glu Gly Trp Val Gln Asn Leu Met Arg Arg Leu Asp Gln	
80 85 90	
CCT CCA GCC CCA GGG AAA CAA AGC CCC GGC TGC AGG AAG AAC CGG GGA	399
Pro Pro Ala Pro Gly Lys Gln Ser Pro Gly Cys Arg Lys Asn Arg Gly	
95 100 105	
ACC TCT AAG TCT GGA AAG AAA GGA AAG GGC TCC AAG GGC TGC AAG AGA	447
Thr Ser Lys Ser Gly Lys Lys Gly Lys Gly Ser Lys Gly Cys Lys Arg	
110 115 120	
ACT GAA CAG ACA CAG CCC TCA AGA GGA TAGCCAGTA GCCCGCCTGG	494
Thr Glu Gln Thr Gln Pro Ser Arg Gly	
125 130	
AGCCCAGGAG ATCCCCCAG AACTTCAAGC TGGGTGGTTC ACGGTCCAAC TCACAGGCAA	554
AGAGGGAGCT AGAAAACAGA CTCAGGAGCC GCTA	588

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 133 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Gln Met Met Thr Leu Ser Leu Leu Ser Leu Val Leu Ala Leu	
1 5 10 15	
Cys Ile Pro Trp Thr Gln Gly Ser Asp Gly Gly Gly Gln Asp Cys Cys	
20 25 30	
Leu Lys Tyr Ser Gln Lys Lys Ile Pro Tyr Ser Ile Val Arg Gly Tyr	
35 40 45	
Arg Lys Gln Glu Pro Ser Leu Gly Cys Pro Ile Pro Ala Ile Leu Phe	
50 55 60	
Ser Pro Arg Lys His Ser Lys Pro Glu Leu Cys Ala Asn Pro Glu Glu	
65 70 75 80	
Gly Trp Val Gln Asn Leu Met Arg Arg Leu Asp Gln Pro Pro Ala Pro	
85 90 95	
Gly Lys Gln Ser Pro Gly Cys Arg Lys Asn Arg Gly Thr Ser Lys Ser	
100 105 110	
Gly Lys Lys Gly Lys Gly Ser Lys Gly Cys Lys Arg Thr Glu Gln Thr	
115 120 125	
Gln Pro Ser Arg Gly	
130	

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 966 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 67..348

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

CTTCCAAGAA GAGCAGCAAA GCTGAAGTAG CAGCAACAGC ACCAGCAGCA ACAGCAAAAA      60
ACAAAC ATG AGT GTG AAG GGC ATG GCT ATA GCC TTG GCT GTG ATA TTG      108
  Met Ser Val Lys Gly Met Ala Ile Ala Leu Ala Val Ile Leu
    1             5             10

TGT GCT ACA GTT GTT CAA GGC TTC CCC ATG TTC AAA AGA GGA CGC TGT      156
Cys Ala Thr Val Val Gln Gly Phe Pro Met Phe Lys Arg Gly Arg Cys
  15             20             25             30

CTT TGC ATA GGC CCT GGG GTA AAA GCA GTG AAA GTG GCA GAT ATT GAG      204
Leu Cys Ile Gly Pro Gly Val Lys Ala Val Lys Val Ala Asp Ile Glu
           35             40             45

AAA GCC TCC ATA ATG TAC CCA AGT AAC AAC TGT GAC AAA ATA GAA GTG      252
Lys Ala Ser Ile Met Tyr Pro Ser Asn Asn Cys Asp Lys Ile Glu Val
           50             55             60

ATT ATT ACC CTG AAA GAA AAT AAA GGA CAA CGA TGC CTA AAT CCC AAA      300
Ile Ile Thr Leu Lys Glu Asn Lys Gly Gln Arg Cys Leu Asn Pro Lys
           65             70             75

TCG AAG CAA GCA AGG CTT ATA ATC AAA AAA GTT GAA AGA AAG AAT TTT      348
Ser Lys Gln Ala Arg Leu Ile Ile Lys Lys Val Glu Arg Lys Asn Phe
  80             85             90

TAAAAATATC AAAACATATG AAGTCCTGGA AAAGGGCATC TGAAAAACCT AGAACAAGTT      408
TAAGTGTGAC TACTGAAATG ACAAGAATTC TACAGTAGGA AACTGAGACT TTTCTATGGT      468
TTTGTGACTT TCAACTTTTG TACAGTTATG TGAAGGATGA AAGGTGGGTG AAAGGACCAA      528
AAACAGAAAT ACAGTCTTCC TGAATGAATG ACAATCAGAA TTCCACTGCC CAAAGGAGTC      588
CAACAATTAA ATGGATTCTT AGGAAAAGCT ACCTTAAGAA AGGCTGGTTA CCATCGGAGT      648
TTACAAAGTG CTTTCACGTT CTTACTTGTT GTATTATACA TTCATGCATT TCTAGGCTAG      708
AGAACCTTCT AGATTTGATG CTTACAATA TTCTGTTGTG ACTATGAGAA CATTTCTGTC      768
TCTAGAAGTT ATCTGTCTGT ATTGATCTTT ATGCTATATT ACTATCTGTG GTTACAGTGG      828
AGACATTGAC ATTATTACTG GAGTCAAGCC CTTATAAGTC AAAAGCACCT ATGTGTCGTA      888

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AAGCATTCCT CAAACATTTA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 948  
 AAAAAAAAAA AAAAAAAAAA 966

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 94 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ser Val Lys Gly Met Ala Ile Ala Leu Ala Val Ile Leu Cys Ala 15  
 1 5 10  
 Thr Val Val Gln Gly Phe Pro Met Phe Lys Arg Gly Arg Cys Leu Cys 30  
 20 25  
 Ile Gly Pro Gly Val Lys Ala Val Lys Val Ala Asp Ile Glu Lys Ala 45  
 35 40  
 Ser Ile Met Tyr Pro Ser Asn Asn Cys Asp Lys Ile Glu Val Ile Ile 60  
 50 55  
 Thr Leu Lys Glu Asn Lys Gly Gln Arg Cys Leu Asn Pro Lys Ser Lys 80  
 65 70 75  
 Gln Ala Arg Leu Ile Ile Lys Lys Val Glu Arg Lys Asn Phe 90  
 85 90

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1354 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

## (ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 75..356

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTCTACTCCT TCCAAGAAGA GCAGCAAAGC TGAAGTAGCA GCAACAGCAC CAGCAGCAAC 60  
 AGCAAAAAAC AAAC ATG AGT GTG AAG GGC ATG GCT ATA GCC TTG GCT GTG 110  
 Met Ser Val Lys Gly Met Ala Ile Ala Leu Ala Val 10  
 1 5  
 ATA TTG TGT GCT ACA GTT GTT CAA GGC TTC CCC ATG TTC AAA AGA GGA 158  
 Ile Leu Cys Ala Thr Val Val Gln Gly Phe Pro Met Phe Lys Arg Gly 25  
 15 20 25

CGC TGT CTT TGC ATA GGC CCT GGG GTA AAA GCA GTG AAA GTG GCA GAT	206
Arg Cys Leu Cys Ile Gly Pro Gly Val Lys Ala Val Lys Val Ala Asp	
30 35 40	
ATT GAG AAA GCC TCC ATA ATG TAC CCA AGT AAC AAC TGT GAC AAA ATA	254
Ile Glu Lys Ala Ser Ile Met Tyr Pro Ser Asn Asn Cys Asp Lys Ile	
45 50 55 60	
GAA GTG ATT ATT ACC CTG AAA GAA AAT AAA GGA CAA CGA TGC CTA AAT	302
Glu Val Ile Ile Thr Leu Lys Glu Asn Lys Gly Gln Arg Cys Leu Asn	
65 70 75	
CCC AAA TCG AAG CAA GCA AGG CTT ATA ATC AAA AAA GTT GAA AGA AAG	350
Pro Lys Ser Lys Gln Ala Arg Leu Ile Ile Lys Lys Val Glu Arg Lys	
80 85 90	
AAT TTT TAAAAATATC AAAACATATG AAGTCCTGGA AAAGGGCATC TGAAAAACCT	406
Asn Phe	
AGAACAAGTT TAACTGTGAC TACTGAAATG ACAAGAATTC TACAGTAGGA AACTGAGACT	466
TTTCTATGGT TTTGTGACTT TCAACTTTTG TACAGTTATG TGAAGGATGA AAGGTGGGTG	526
AAAGGACCAA AAACAGAAAT ACAGTCTTCC TGAATGAATG ACAATCAGAA TTCCACTGCC	586
CAAAGGAGTC CAACAATTAA ATGGATTTCT AGGAAAAGCT ACCTTAAGAA AGGCTGGTTA	646
CCATCGGAGT TTACAAAGTG CTTTCACGTT CTTACTTGTT GTATTATACA TTATGCAATT	706
TCTAGGCTAG AGAACCTTCT AGATTTGATG CTTACAATA TTCTGTTGTG ACTATGAGAA	766
CATTTCTGTC TCTAGAAGTT ATCTGTCTGT ATTGATCTTT ATGCTATATT ACTATCTGTG	826
GTTACAGTGG AGACATTGAC ATTATTACTG GAGTCAAGCC CTTATAAGTC AAAAGCACCT	886
ATGTGTCGTA AAGCATTCTT CAAACATTTT TTCATGCAAA TACACACTTC TTTCCCCAAA	946
TATCATGTAG CACATCAATA TGTAGGGAAA CATTCCTTATG CATCATTTGG TTTGTTTTAT	1006
AACCAATTCA TTAAATGTAA TTCATAAAAT GTACTATGAA AAAAATTATA CGCTATGGGA	1066
TACTGGCAAC AGTGCACATA TTTCATAACC AAATTAGCAG CACCGGTCTT AATTTGATGT	1126
TTTTCAACTT TTATTCATTG AGATGTTTTG AAGCAATTAG GATATGTGTG TTTACTGTAC	1186
TTTTTGTTTT GATCCGTTTG TATAAATGAT AGCAATATCT TGGACACATT TGAAATACAA	1246
AATGTTTTTG TCTACCAAAG AAAAATGTTG AAAAATAAGC AAATGTATAC CTAGCAATCA	1306
CTTTTACTTT TTGTAATTCT GTCTCTTAGA AAAATACATA ATCTAATT	1354

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 94 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ser Val Lys Gly Met Ala Ile Ala Leu Ala Val Ile Leu Cys Ala  
 1 5 10 15  
 Thr Val Val Gln Gly Phe Pro Met Phe Lys Arg Gly Arg Cys Leu Cys  
 20 25 30  
 Ile Gly Pro Gly Val Lys Ala Val Lys Val Ala Asp Ile Glu Lys Ala  
 35 40 45  
 Ser Ile Met Tyr Pro Ser Asn Asn Cys Asp Lys Ile Glu Val Ile Ile  
 50 55 60  
 Thr Leu Lys Glu Asn Lys Gly Gln Arg Cys Leu Asn Pro Lys Ser Lys  
 65 70 75 80  
 Gln Ala Arg Leu Ile Ile Lys Lys Val Glu Arg Lys Asn Phe  
 85 90

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 813 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 86..544

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGGAAGATAC ATTCACAGAA AGAGCTTCCT GCACAAAGTA AGCCACCAGC GCAACATGAC 60  
 AGTGAAGACC CTGCATGGCC CAGCC ATG GTC AAG TAC TTG CTG CTG TCG ATA 112  
 Met Val Lys Tyr Leu Leu Leu Ser Ile  
 1 5  
 TTG GGG CTT GCC TTT CTG AGT GAG GCG GCA GCT CGG AAA ATC CCC AAA 160  
 Leu Gly Leu Ala Phe Leu Ser Glu Ala Ala Ala Arg Lys Ile Pro Lys  
 10 15 20 25  
 GTA GGA CAT ACT TTT TTC CAA AAG CCT GAG AGT TGC CCG CCT GTG CCA 208  
 Val Gly His Thr Phe Phe Gln Lys Pro Glu Ser Cys Pro Pro Val Pro  
 30 35 40  
 GGA GGT AGT ATG AAG CTT GAC ATT GGC ATC ATC AAT GAA AAC CAG CGC 256  
 Gly Gly Ser Met Lys Leu Asp Ile Gly Ile Ile Asn Glu Asn Gln Arg  
 45 50 55  
 GTT TCC ATG TCA CGT AAC ATC GAG AGC CGC TCC ACC TCC CCC TGG AAT 304  
 Val Ser Met Ser Arg Asn Ile Glu Ser Arg Ser Thr Ser Pro Trp Asn  
 60 65 70  
 TAC ACT GTC ACT TGG GAC CCC AAC CGG TAC CCC TCG GAA GTT GTA CAG 352  
 Tyr Thr Val Thr Trp Asp Pro Asn Arg Tyr Pro Ser Glu Val Val Gln  
 75 80 85

GCC CAG TGT AGG AAC TTG GGC TGC ATC AAT GCT CAA GGA AAG GAA GAC 400  
 Ala Gln Cys Arg Asn Leu Gly Cys Ile Asn Ala Gln Gly Lys Glu Asp  
 90 95 100 105  
 ATC TCC ATG AAT TCC GTT CCC ATC CAG CAA GAG ACC CTG GTC GTC CGG 448  
 Ile Ser Met Asn Ser Val Pro Ile Gln Gln Glu Thr Leu Val Val Arg  
 110 115 120  
 AGG AAG CAC CAA GGC TGC TCT GTT TCT TTC CAG TTG GAG AAG GTG CTG 496  
 Arg Lys His Gln Gly Cys Ser Val Ser Phe Gln Leu Glu Lys Val Leu  
 125 130 135  
 GTG ACT GTT GGC TGC ACC TGC GTC ACC CCT GTC ATC CAC CAT GTG CAG 544  
 Val Thr Val Gly Cys Thr Cys Val Thr Pro Val Ile His His Val Gln  
 140 145 150  
 TAAGAGGTGC ATATCCACTC AGCTGAAGAA GCTGTAGAAA TGCCACTCCT TACCCAGTGC 604  
 TCTGCAACAA GTCCTGTCTG ACCCCCAATT CCCTCCACTT CACAGGACTC TTAATAAGAC 664  
 CTGCACGGAT GGAAACAGAA AATATTCACA ATGTATGTGT GTATGTACTA CACTTTATAT 724  
 TTGATATCTA AAATGTTAGG AGAAAAATTA ATATATTCAG TGCTAATATA ATAAAGTATT 784  
 AATAATTTAA AAATAAAAAA AAAAAAAAAA 813

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 153 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Val Lys Tyr Leu Leu Leu Ser Ile Leu Gly Leu Ala Phe Leu Ser  
 1 5 10 15  
 Glu Ala Ala Ala Arg Lys Ile Pro Lys Val Gly His Thr Phe Phe Gln  
 20 25 30  
 Lys Pro Glu Ser Cys Pro Pro Val Pro Gly Gly Ser Met Lys Leu Asp  
 35 40 45  
 Ile Gly Ile Ile Asn Glu Asn Gln Arg Val Ser Met Ser Arg Asn Ile  
 50 55 60  
 Glu Ser Arg Ser Thr Ser Pro Trp Asn Tyr Thr Val Thr Trp Asp Pro  
 65 70 75 80  
 Asn Arg Tyr Pro Ser Glu Val Val Gln Ala Gln Cys Arg Asn Leu Gly  
 85 90 95  
 Cys Ile Asn Ala Gln Gly Lys Glu Asp Ile Ser Met Asn Ser Val Pro  
 100 105 110  
 Ile Gln Gln Glu Thr Leu Val Val Arg Arg Lys His Gln Gly Cys Ser  
 115 120 125  
 Val Ser Phe Gln Leu Glu Lys Val Leu Val Thr Val Gly Cys Thr Cys

130

135

140

Val Thr Pro Val Ile His His Val Gln  
145 150

What is claimed is:

1. A composition comprising an isolated polynucleotide selected from the group consisting of:
  - (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 38 to nucleotide 1447;
  - (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:1 encoding a protein having biological activity;
  - (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2;
  - (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity;
  - (e) a polynucleotide which is an allelic variant of SEQ ID NO:1;and
  - (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).
2. A composition of claim 1 wherein said polynucleotide is operably linked to an expression control sequence.
3. A host cell transformed with a composition of claim 2.
4. The host cell of claim 3, wherein said cell is a mammalian cell.
5. A process for producing a protein, which comprises:
  - (a) growing a culture of the host cell of claim 3 in a suitable culture medium; and
  - (b) purifying the protein from the culture
6. A protein produced according to the process of claim 5.
7. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:2; and
  - (b) fragments of the amino acid sequence of SEQ ID NO:2;
- the protein being substantially free from other mammalian proteins.

8. The composition of claim 7, further comprising a pharmaceutically acceptable carrier.

9. A composition comprising an antibody which specifically reacts with the protein of claim 7.

10. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 8.

11. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 52 to nucleotide 2034;

- (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:3 encoding a protein having biological activity;

- (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:4;

- (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity;

- (e) a polynucleotide which is an allelic variant of SEQ ID NO:4;

and

- (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

12. A composition of claim 11 wherein said polynucleotide is operably linked to an expression control sequence.

13. A host cell transformed with a composition of claim 12.
14. The host cell of claim 13, wherein said cell is a mammalian cell.
15. A process for producing a protein, which comprises:
  - (a) growing a culture of the host cell of claim 13 in a suitable culture medium; and
  - (b) purifying the protein from the culture
16. A protein produced according to the process of claim 15.
17. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:
  - (a) the amino acid sequence of SEQ ID NO:4; and
  - (b) fragments of the amino acid sequence of SEQ ID NO:4;the protein being substantially free from other mammalian proteins.
18. The composition of claim 17, further comprising a pharmaceutically acceptable carrier.
19. A composition comprising an antibody which specifically reacts with the protein of claim 17.
20. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 18.
21. A composition comprising an isolated polynucleotide selected from the group consisting of:
  - (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 76 to nucleotide 474;



- (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:5 encoding a protein having biological activity;
- (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6;
- (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity;
- (e) a polynucleotide which is an allelic variant of SEQ ID NO:5;
- and
- (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

22. A composition of claim 21 wherein said polynucleotide is operably linked to an expression control sequence.

23. A host cell transformed with a composition of claim 22.

24. The host cell of claim 23, wherein said cell is a mammalian cell.

25. A process for producing a protein, which comprises:

- (a) growing a culture of the host cell of claim 23 in a suitable culture medium; and
- (b) purifying the protein from the culture

26. A protein produced according to the process of claim 25.

27. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:6; and
  - (b) fragments of the amino acid sequence of SEQ ID NO:6;
- the protein being substantially free from other mammalian proteins.

28. The composition of claim 27, further comprising a pharmaceutically acceptable carrier.

29. A composition comprising an antibody which specifically reacts with the protein of claim 27.

30. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 28.

31. A composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 67 to nucleotide 348;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:7 encoding a protein having biological activity;

(c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:8;

(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 having biological activity;

(e) a polynucleotide which is an allelic variant of SEQ ID NO:7; and

(f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

32. A composition of claim 31 wherein said polynucleotide is operably linked to an expression control sequence.

33. A host cell transformed with a composition of claim 32.

34. The host cell of claim 33, wherein said cell is a mammalian cell.

35. A process for producing a protein, which comprises:
- (a) growing a culture of the host cell of claim 33 in a suitable culture medium; and
  - (b) purifying the protein from the culture
36. A protein produced according to the process of claim 35.
37. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:
- (a) the amino acid sequence of SEQ ID NO:8; and
  - (b) fragments of the amino acid sequence of SEQ ID NO:8;
- the protein being substantially free from other mammalian proteins.
38. The composition of claim 37, further comprising a pharmaceutically acceptable carrier.
39. A composition comprising an antibody which specifically reacts with the protein of claim 37.
40. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 38.
41. A composition comprising an isolated polynucleotide selected from the group consisting of:
- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 75 to nucleotide 356;
  - (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:9 encoding a protein having biological activity;
  - (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:10;

- (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity;
- (e) a polynucleotide which is an allelic variant of SEQ ID NO:9;
- and
- (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

42. A composition of claim 41 wherein said polynucleotide is operably linked to an expression control sequence.

43. A host cell transformed with a composition of claim 42.

44. The host cell of claim 43, wherein said cell is a mammalian cell.

45. A process for producing a protein, which comprises:

- (a) growing a culture of the host cell of claim 43 in a suitable culture medium; and
- (b) purifying the protein from the culture

46. A protein produced according to the process of claim 45.

47. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:10; and
  - (b) fragments of the amino acid sequence of SEQ ID NO:10;
- the protein being substantially free from other mammalian proteins.

48. The composition of claim 47, further comprising a pharmaceutically acceptable carrier.

49. A composition comprising an antibody which specifically reacts with the protein of claim 47.

50. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 48.

51. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 86 to nucleotide 544;
- (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:11 encoding a protein having biological activity;
- (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:12;
- (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity;
- (e) a polynucleotide which is an allelic variant of SEQ ID NO:11; and
- (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

52. A composition of claim 51 wherein said polynucleotide is operably linked to an expression control sequence.

53. A host cell transformed with a composition of claim 52.

54. The host cell of claim 53, wherein said cell is a mammalian cell.

55. A process for producing a protein, which comprises:

- (a) growing a culture of the host cell of claim 53 in a suitable culture medium; and
- (b) purifying the protein from the culture

56. A protein produced according to the process of claim 55.

57. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:12; and
  - (b) fragments of the amino acid sequence of SEQ ID NO:12;
- the protein being substantially free from other mammalian proteins.

58. The composition of claim 57, further comprising a pharmaceutically acceptable carrier.

59. A composition comprising an antibody which specifically reacts with the protein of claim 57.

60. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 58.



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> C12N 15/12, 15/19, C07K 14/47, 14/52 C12N 5/10, A61K 38/17, C07K 16/18	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 97/07198</b> <b>(43) International Publication Date:</b> 27 February 1997 (27.02.97)
<b>(21) International Application Number:</b> PCT/US96/12897 <b>(22) International Filing Date:</b> 8 August 1996 (08.08.96)  <b>(30) Priority Data:</b> Not furnished 11 August 1995 (11.08.95) US  <b>(71) Applicant:</b> GENETICS INSTITUTE, INC. [US/US]; 87 CambridgePark Drive, Cambridge, MA 02140 (US).  <b>(72) Inventors:</b> JACOBS, Kenneth; 151 Beaumont Avenue, Newton, MA 02160 (US). MCCOY, John, M.; 63 Pine Ridge Road, Reading, MA 01867 (US). KELLEHER, Kerry; 50 Hurley Circle, Marlborough, MA 01752 (US). CARLIN, McKeough; 16 Chauncy Street #22, Cambridge, MA 02138 (US).  <b>(74) Agent:</b> BROWN, Scott, A.; Genetics Institute, Inc., Legal Affairs, 87 CambridgePark Drive, Cambridge, MA 02140 (US).		<b>(81) Designated States:</b> AU, CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>  <b>(88) Date of publication of the international search report:</b> 24 July 1997 (24.07.97)
<b>(54) Title:</b> DNA SEQUENCES AND SECRETED PROTEINS ENCODED THEREBY  <b>(57) Abstract</b>  Novel polynucleotides and the proteins encoded thereby are disclosed.		

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# INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/US 96/12897

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C12N15/19 C07K14/47 C07K14/52 C12N5/10  
A61K38/17 C07K16/18

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>THE JOURNAL OF IMMUNOLOGY, vol. 154, no. 7, 1 April 1995, pages 3333-3340, XP002032346 MIYAKE ET AL.: "RP105, a novel B cell surface molecule implicated in B cell activation, is a member of the Leucine-rich repeat protein family" see abstract see page 3336; figure 2 see page 3337, left-hand column, paragraph 2 see page 3338; figure 4 --- -/--</p>	11-19

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
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- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*A\* document member of the same patent family

Date of the actual completion of the international search

5 June 1997

Date of mailing of the international search report

13. 06. 97

Name and mailing address of the ISA

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# INTERNATIONAL SEARCH REPORT

Int'l Application No  
PCT/US 96/12897

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>DATABASE EMBL ENTRY MM04637 Accession Number W67046, 15 June 1996 MARRA ET AL.: "The WashU-HHMI mouse EST project" XP002032347 see abstract &amp; Unpublished</p>	21-29
P,X	<p style="text-align: center;">---</p> <p>DATABASE EMBL ENTRY MM3831 Accession Number W08383, 27 April 1996 MARRA ET AL.: "The WashU-HHMI mouse EST project" XP002032348 see abstract &amp; Unpublished</p>	21-29
P,X	<p style="text-align: center;">---</p> <p>DATABASE EMBL ENTRY MM93010 Accession Number W17930, 4 May 1996 MARRA ET AL.: "The WashU-HHMI mouse EST project" XP002032349 see abstract &amp; Unpublished</p>	21-29
P,X	<p style="text-align: center;">---</p> <p>DATABASE EMBL ENTRY MM1705 Accession number W11170, 29 April 1996 MARRA ET AL.: "The WashU-HHMI mouse EST project" XP002032350 see abstract &amp; Unpublished</p>	21-24
P,X	<p style="text-align: center;">---</p> <p>DATABASE EMBL ENTRY HS309B01B Accession number D62634, 29 August 1995 FUJIWARA ET AL.: XP002032351 see abstract &amp; Unpublished</p>	41-44
E	<p style="text-align: center;">---</p> <p>WO 97 04097 A (GENETICS INST) 6 February 1997 Seq.ID:1/2 see page 33 - page 34 see page 39 - page 42; claims</p>	51-60
A	<p style="text-align: center;">---</p> <p>WO 95 18826 A (SCHERING CORP ;INST NAT SANTE RECH MED (FR)) 13 July 1995 see page 50 - page 63</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	51-60

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 96/12897

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 914 025 A (MANOIL COLIN ET AL) 3 April 1990 see the whole document ---	1-10
A	JOURNAL OF BACTERIOLOGY, vol. 177, no. 1, 1 January 1995, pages 59-65, XP000560419 ENG MONG LIM ET AL: "IDENTIFICATION OF MYCOBACTERIUM TUBERCULOSIS DNA SEQUENCES ENCODING EXPORTED PROTEINS BY USING PHOA GENE FUSIONS" ---	
A	JOURNAL OF CELLULAR BIOCHEMISTRY, vol. 21A, 10 March 1995 - 4 April 1995, page 19 XP002027246 JACOBS ET AL.: "A novel method for isolating eukaryotic cDNA clones encoding secreted proteins" ---	
T	US 5 536 637 A (JACOBS KENNETH) 16 July 1996 -----	

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/12897

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 10, 20, 30, 40, 50, 60  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although these claims are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

5 inventions \* see continuation-sheet PCT/ISA/210 \*

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION C NTINUED FROM PCT/ISA/210**

1) claims 1-10 totally.

Nucleotide sequence as in Seq.ID:1 encoding polypeptide as in Seq.ID:2, fragments, compositions and potential therapeutical uses.

2) claims 11-20 totally.

Nucleotide sequence as in Seq.ID:3 encoding polypeptide as in Seq.ID:4, fragments, compositions and potential therapeutical uses.

3) claims 21-30 totally.

Nucleotide sequence as in Seq.ID:5 encoding polypeptide as in Seq.ID:6, fragments, compositions and potential therapeutical uses.

4) claims 31-50 totally.

Nucleotide sequence as in Seq.ID:7/9 encoding polypeptide as in Seq.ID:8/10, fragments, compositions and potential therapeutical uses.

5) claims 51-60 totally.

Nucleotide sequence as in Seq.ID:11 encoding polypeptide as in Seq.ID:12, fragments, compositions and potential therapeutical uses.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/12897

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9704097 A	06-02-97	AU 6712396 A AU 6768596 A WO 9707198 A	18-02-97 12-03-97 27-02-97
WO 9518826 A	13-07-95	AU 1520895 A EP 0733069 A JP 9501572 T	01-08-95 25-09-96 18-02-97
US 4914025 A	03-04-90	NONE	
US 5536637 A	16-07-96	NONE	